GENETIC EVALUATION OF
IN BACTERIAL
REVERSE MUTATION ASSAYS

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This summary of data and conclusions is based upon the sample received. Additional studies may be required as specific uses and formulations are developed or if process changes occur.

ABSTRACT

The test material was evaluated for genetic activity in the Salmonella typhimurium and Escherichia coli Reverse Mutation assays as outlined in "O.E.C.D. Guidelines for Testing of Chemicals" - Draft Protocol Nos. 419 and 420. Concentrations above 312.5 µg/plate were generally toxic to the tester strains. No evidence of genetic activity was observed.

*Amine siloxane hydrolyzate

Distribution

TABLE OF CONTENTS

OBJECTIVE

The objective of this study was to evaluate the test material for genetic activity in the Salmonella typhimurium and Escherichia coli Reverse Mutation Assays as outlined in O.E.C.D. GUIDELINES FOR TESTING OF CHEMICALS (CRF 46 (162)/8-21-81) as part of the Minimum Premarket Data Set for new chemicals.

MATERIALS

A. Test Material

B. Indicator Microorganisms

Salmonella typhimurium, str.	TA-1535	TA-98
	TA-97	TA-100
Escherichia coli, str.	WP2	

C Activation System

Bacteria were exposed to the test substance both in the presence and absence of a mammalian activation mixture (S-9 mix) prepared in accordance with published protocols (Ames, et al., 1975; Matsushima, et al., 1976).

1. Component

Component	Final Concentration/ml
$MgCl_2$	8 µ moles
KCl	33 µ moles
NADP	4 µ moles
Glucose-6-phosphate	5 µ moles
Sodium phosphate, pH 7.4	100 µ moles
Homogenate fraction equivalent to 25 mg of wet tissue	

2. S-9 Homogenate

A 9000 x g supernatant prepared from Sprague-Dawley adult male rat liver induced by AROCLOR 1254 five days prior to kill. Purchased from Litton-Bionetics, Inc., Kensington, Maryland. Stored until use at $-76^{o}C$.

D. Positive Control Chemicals

Chemicals used for positive controls in the non-activation and activation assays.

Assay	Chemical*	Solvent**
Non-activation	Sodium Azide (AZ)	Water or Saline
	9-Amino Acridine (AA)	Ethanol
	Daunomycin (D)	Water
	N-Methyl-N-nitro-N-nitrosoquanidine (MNNG)	Water
Activation	2-Anthramine (ANTH)	Dimethylsulfoxide

*Concentrations given in Results section.
**Previously shown to be non-mutagenic.

E. Solvent

Dimethylsulfoxide (DMSO) was used to prepare dilutions of the test material. The solvent employed and concentrations of chemicals are recorded in the Results section.

EXPERIMENTAL DESIGN

A. Principle of the Test Method

Bacteria are exposed to test chemical with and without metabolic activation and plated on minimal medium. After a suitable period of incubation, revertant colonies are counted and compared to the number of spontaneous revertants in an untreated (solvent) control culture. Positive and negative (solvent) controls are included in each experiment.

B. Description of the Test Method

Five different amounts of test chemical separated by half-log intervals were tested (de Serres and Shelby, 1979). Substances were tested up to the limit of solubility or toxicity. Toxicity may be evidenced by a reduction in the number of spontaneous revertants, a clearing of the background lawn or by degree of survival of treated cultures. Nontoxic chemicals are tested to 5 mg/plate before considering the test substance negative.

Plates were incubated for 72 hours at 37°C.

1. Direct Plate Incorporation Method

Nonactivation Assay

To a sterile 13 x 100 mm test tube placed in a 43°C water bath, the following is added in order:

. 2.00 ml of 0.6% agar containing:

0.05 mM histidine and 0.05 mM biotin (Salmonella Assay)
0.05 mM tryptophan (Escherichia coli Assay)

. 0.05 ml of a solution of the test chemical to give the appropriate dose.

. 0.01 ml - 0.2 ml of indicator organism/s.

. 0.50 ml of 0.2M phosphate buffer, pH 7.4.

This mixture is swirled gently and then poured over the surface of minimal agar plates. After the top agar has set, the plates are incubated at 37°C for 72 hours. The number of revertant colonies growing in the plates is counted and recorded.

Activation Assay

The activation assay is run concurrently with the nonactivation assay. The only difference is the addition of 0.5 ml of S-9 mix to the tubes in place of 0.5 ml of phosphate buffer which is added in nonactivation assays. All other details are similar to the procedure for nonactivation assays.

All plating was done in triplicate.

RESULTS

A. Spot Plate Test
The test material was negative against all testor strains, with and without metabolic activation.

B. Overlay Plate Test

See attached Tables I-V for results. Solvent of choice and concentrations of material tested are given in the tables. No evidence of mutagenic potential was observed.

DATA

A. Data Presentation

The data are presented as the number of revertant colonies per plate. The number of revertant colonies on both negative (solvent) and positive control plates are also presented.

Individual plate counts, the mean number of revertant colonies per plate and standard deviation are presented for test chemical and positive and negative (solvent) controls.

B. Statistical Evaluation

Data was evaluated using appropriate statistical methods.

C. Results

Because the test article and the cells are incubated in the overlay for approximately two days and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.

The combined incubation of the test article and the cells in the overlay permits constant exposure of the indicator cells for approximately two days.

1. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test article, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol employs several doses ranging over two or three log concentrations.

2. Dose-Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test article may kill any mutants that are induced, and the test article will not appear to be mutagenic.

3. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test article solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data is compared. The positive control assay will be conducted to demonstrate that the test systems are functional with known mutagens.

4. Evaluation Criteria for Toxicity

Complete Toxicity

When there are no revertants observed on the plate(s) treated with the test compound, the test compound will be defined as toxic to all or any of the indicator strains at that (those) particular dose(s).

Slight Toxicity

When there are fifty percent or less number of revertants on the plate(s) treated with the test compound as compared to the solvent plate(s), the test compound will be defined as slightly toxic to all or any of the indicator strains at that (those) particular dose(s).

5. Evaluation Criteria for Ames Assay

Because the procedures to be used to evaluate the mutagenicity of the test article are semiquantitative, the criteria to be used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the criteria established by K. C. Chu, et al., (1981), Mutation Res., 119-132.

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

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This report constituted of pages 1-10, and Tables I-V, signed this 8th day of April, 1985.

Authors:

Approved By:

Typed By:

QUALITY ASSURANCE STATEMENT

This report represents data generated by the Toxicology Department, This study was conducted according to EPA Toxic Substances Control; Good Laboratory Practices Regulations; 40 CFR, Part 797, Vol. 48, No. 230. The results reported accurately reflect the data generated. All raw data is located at

Study Started:	January 29, 1985
Study Completed:	February 1, 1985
Date Audited:	January 29, 1985 and February 1, 1985
Report Issued:	April 16, 1985

April 8, 1985
Date:

TABLE IA

TEST MATERIAL :

PREPARED BY:

INITIATION DATE: 1-29-85

NONACTIVATION TEST--TA-1535

REVERTANTS PER PLATE	SOLV CON	POS* CON	CONC.OF TEST COMPOUND (UG/PLATE) 312.5	625.0	1250	2500	5000
PLATE 1	21	262	10	3	0	0	0
PLATE 2	20	254	12	5	0	0	0
PLATE 3	20	278	11	4	0	0	0
MEAN	20	264	11	4	0	0	0
S.D.	0	12	1	1	0	0	0

ACTIVATION TEST--TA-1535

PLATE 1	23	487	23	30	6	5	2
PLATE 2	20	512	20	24	3	2	1
PLATE 3	21	467	26	27	3	1	1
MEAN	21	488	23	27	4	2	1
S.D.	1	22	3	3	1	2	0

* NONACTIVATION	AZ	10	UG/PLATE
ACTIVATION	ANTH	10	UG/PLATE
SOLVENT	DMSO	50	UL/PLATE

TABLE IB

TEST MATERIAL : PREPARED BY:
INITIATION DATE: 2-5-85

NONACTIVATION TEST--TA-1535

REVERTANTS PER PLATE	SOLV CON	POS* CON	CONC.OF TEST COMPOUND (UG/PLATE) 19.5	39.0	78.1	156.2	312.5
PLATE 1	19	212	17	20	16	18	16
PLATE 2	19	198	19	15	16	21	15
PLATE 3	20	204	16	18	20	17	14
MEAN	19	204	17	17	17	18	15
S.D.	0	7	1	2	2	2	1

ACTIVATION TEST--TA-1535

PLATE 1	21	456	24	23	20	22	18
PLATE 2	23	467	24	24	19	16	15
PLATE 3	24	412	19	26	17	17	18
MEAN	22	445	22	24	18	18	17
S.D.	1	29	2	1	1	3	1

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* NONACTIVATION AZ 10 UG/PLATE
ACTIVATION ANTH 10 UG/PLATE
SOLVENT DMSO 50 UL/PLATE

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TABLE IIA

TEST MATERIAL : PREPARED BY:
INITIATION DATE: 1-29-85

NONACTIVATION TEST--TA-97

REVERTANTS PER PLATE	SOLV CON	POS* CON	CONC.OF TEST COMPOUND (UG/PLATE) 312.5	625.0	1250	2500	5000
PLATE 1	138	396	120	0	0	0	0
PLATE 2	121	411	118	0	0	0	0
PLATE 3	126	417	131	0	0	0	0
MEAN	128	408	123	0	0	0	0
S.D.	8	10	7	0	0	0	0

ACTIVATION TEST--TA-97

PLATE 1	126	789	133	127	118	40	0
PLATE 2	124	810	128	131	122	54	2
PLATE 3	133	816	120	128	116	43	0
MEAN	127	805	127	128	118	45	0
S.D.	4	14	6	2	3	7	1

* NONACTIVATION NQNO 10 UG/PLATE
ACTIVATION AF 10 UG/PLATE
SOLVENT DMSO 50 UL/PLATE

TABLE IIB

TEST MATERIAL :

PREPARED BY:

INITIATION DATE: 2-5-85

NONACTIVATION TEST--TA-97

REVERTANTS PER PLATE	SOLV CON	POS* CON	CONC. OF TEST COMPOUND (UG/PLATE) 19.5	39.0	78.1	156.2	312.5
PLATE 1	120	493	130	138	126	117	117
PLATE 2	124	476	136	127	124	130	119
PLATE 3	129	474	140	130	124	122	116
MEAN	124	481	135	131	124	123	117
S.D.	4	10	5	5	1	6	1

ACTIVATION TEST--TA-97

PLATE 1	127	852	136	127	119	111	123
PLATE 2	133	871	128	134	124	120	125
PLATE 3	129	912	120	130	126	118	130
MEAN	129	878	128	130	123	116	126
S.D.	3	30	8	3	3	4	3

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* NONACTIVATION	NQNO	10	UG/PLATE
ACTIVATION	AF	10	UG/PLATE
SOLVENT	DMSO	50	UL/PLATE

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TABLE IIIA

TEST MATERIAL :

PREPARED BY:

INITIATION DATE: 1-29-85

NONACTIVATION TEST--TA-98

REVERTANTS PER PLATE	SOLV CON	POS* CON	CONC. OF TEST COMPOUND (UG/PLATE) 312.5	625.0	1250	2500	5000
PLATE 1	29	212	4	0	0	0	0
PLATE 2	31	228	2	0	0	0	0
PLATE 3	24	241	2	0	0	0	0
MEAN	28	227	2	0	0	0	0
S.D.	3	14	1	0	0	0	0

ACTIVATION TEST--TA-98

PLATE 1	38	927	37	20	12	0	0
PLATE 2	42	1000	40	28	17	0	0
PLATE 3	40	875	33	24	19	0	0
MEAN	40	934	36	24	16	0	0
S.D.	2	62	3	4	3	0	0

* NONACTIVATION D 10 UG/PLATE
ACTIVATION AF 10. UG/PLATE
SOLVENT DMSO 50 UL/PLATE

TABLE IIIB

TEST MATERIAL : PREPARED BY:

INITIATION DATE: 2-5-85

NONACTIVATION TEST--TA-98

REVERTANTS PER PLATE	SOLV CON	POS* CON	CONC. OF TEST COMPOUND (UG/PLATE) 19.5	39.0	78.1	156.2	312.5
PLATE 1	33	229	41	40	32	41	29
PLATE 2	41	237	42	38	30	33	30
PLATE 3	32	212	42	36	32	37	32
MEAN	35	226	41	38	31	37	30
S.D.	4	12	0	2	1	4	1

ACTIVATION TEST--TA-98

PLATE 1	44	1000	38	37	41	36	28
PLATE 2	41	1000	36	37	40	38	26
PLATE 3	45	1000	42	33	31	29	26
MEAN	43	1000	38	35	37	34	26
S.D.	2	0	3	2	5	4	1

* NONACTIVATION D 10 UG/PLATE
ACTIVATION AF 10 UG/PLATE
SOLVENT DMSO 50 UL/PLATE

TABLE IVA

TEST MATERIAL : PREPARED BY:

INITIATION DATE: 1-29-85

NONACTIVATION TEST--TA-100

REVERTANTS PER PLATE	SOLV CON	POS* CON	CONC.OF TEST COMPOUND (UG/PLATE) 312.5	625.0	1250	2500	5000
PLATE 1	153	603	37	2	0	0	0
PLATE 2	161	598	32	1	0	0	0
PLATE 3	152	633	28	3	0	0	0
MEAN	155	611	32	2	0	0	0
S.D.	4	18	4	1	0	0	0

ACTIVATION TEST--TA-100

PLATE 1	160	854	104	41	30	0	0
PLATE 2	154	1000	98	38	27	0	0
PLATE 3	161	1000	101	36	24	0	0
MEAN	158	951	101	38	27	0	0
S.D.	3	84	3	2	3	0	0

* NONACTIVATION AZ 10 UG/PLATE
ACTIVATION AF 10 UG/PLATE
SOLVENT DMSO 50 UL/PLATE

TABLE IVB

TEST MATERIAL : PREPARED BY:

INITIATION DATE: 2-5-85

NONACTIVATION TEST--TA-100

REVERTANTS PER PLATE	SOLV CON	POS* CON	CONC. OF TEST COMPOUND (UG/PLATE) 19.5	39.0	78.1	156.2	312.5
PLATE 1	144	576	128	171	162	139	121
PLATE 2	151	562	147	134	172	147	133
PLATE 3	147	612	159	144	153	126	137
MEAN	147	583	144	149	162	137	130
S.D.	3	25	15	19	9	10	8

ACTIVATION TEST--TA-100

PLATE 1	172	1000	181	166	140	168	149
PLATE 2	164	1000	176	120	162	155	138
PLATE 3	162	1000	184	165	155	152	137
MEAN	166	1000	180	150	152	158	141
S.D.	5	0	4	26	11	8	6

* NONACTIVATION AZ 10 UG/PLATE
ACTIVATION AF 10 UG/PLATE
SOLVENT DMSO 50 UL/PLATE

TABLE VA

TEST MATERIAL : PREPARED BY:
INITIATION DATE: 1-29-85

NONACTIVATION TEST--WP2

REVERTANTS PER PLATE	SOLV CON	POS* CON	CONC.OF TEST COMPOUND (UG/PLATE) 312.5	625.0	1250	2500	5000
PLATE 1	14	400	45	42	29	15	0
PLATE 2	32	412	36	41	30	12	0
PLATE 3	30	433	32	34	22	11	1
MEAN	25	415	37	39	27	12	0
S.D.	9	16	6	4	4	2	0

ACTIVATION TEST--WP2

PLATE 1	25	501	31	39	33	30	18
PLATE 2	28	497	32	40	28	32	20
PLATE 3	28	486	28	33	30	32	21
MEAN	27	494	30	37	30	31	19
S.D.	1	7	2	3	2	1	1

* NONACTIVATION MNNG 10 UG/PLATE
ACTIVATION ANTH 10 UG/PLATE
SOLVENT DMSO 50 UL/PLATE

TABLE VB

TEST MATERIAL :

PREPARED BY:

INITIATION DATE: 2-5-85

NONACTIVATION TEST--WP2

REVERTANTS PER PLATE	SOLV CON	POS* CON	CONC.OF TEST COMPOUND (UG/PLATE) 19.5	39.0	78.1	156.2	312.5
PLATE 1	22	322	20	24	17	30	26
PLATE 2	19	417	19	24	22	31	19
PLATE 3	24	338	20	26	20	24	22
MEAN	21	359	19	24	19	28	22
S.D.	2	50	0	1	2	3	3

ACTIVATION TEST--WP2

PLATE 1	30	476	41	40	25	34	22
PLATE 2	28	501	28	36	30	24	29
PLATE 3	25	433	40	31	30	20	27
MEAN	27	470	36	35	28	26	26
S.D.	2	34	7	4	2	7	3

* NONACTIVATION MNNG 10 UG/PLATE
ACTIVATION ANTH 10 UG/PLATE
SOLVENT DMSO 50 UL/PLATE